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RESEARCH ARTICLE

Studies on Fungal Aerospores as Potential Aeroallergens in Gondia, Maharashtra

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ABSTRACT

The present study was undertaken to determine the impact of bioaerosol contamination in air and analyse functional relationships between composition of airspora and meteorological factors. Pearson correlation coefficient test relationship between bioaerosols and temperature, relative humidity and relative rains and two ways and the effect of different seasons and sampling stations on the bioaerosol contamination of air. The abundance of airborne spores shows distinct temporal patterns that persist across a mosaic of natural vegetation. Peak spore loads found during the Kharif season and lower spore abundance during Rabi season. Across spatial and temporal dimensions, microclimate is a stronger indicator of airborne spore concentration than vegetation type.

KEY WORDS: Aerospore, allergens, fungal, meteorological, pollution

INTRODUCTION

There are about 1.5 million fungal species reported in the world so far, and about 110 of them have been linked to allergies related to the respiratory system. Molds and yeasts are the two kinds of fungi that are distinguished from one another by their morphological structures. The atmosphere contains both yeast spores and molds, both of which can lead to allergic reactions (Kurup, 2000; Bush & Portnoy, 2001). Indoor and outdoor fungi are the two categories of which inhaled fungal allergens are divided. The fungal spores present in both interior and exterior of the house, in the event of constant air movement. Building interiors can harbour wide variety of fungal species, those are typically found outside belong to the following genera: *Penicillium*, *Cladosporium*, *Aspergillus*, *Ulocladium*, *Aureobasidium*, *Alternaria*, *Phoma*, *Nigrospora*, *Rhizopus*, *Mucor*, *Epicoccum*, *Stemphylium*, *Curvularia*, *Fusarium*, *Scopulariopsis*, *Cephalosporium*, *Trichoderma*, *Chaetomium*, *Streptomyces*, *Candida*, *Cryptococcus*, and *Rhodotorula* (Chao *et al*., 2002; Wargocki *et al*., 2002). Fungal spore sizes can vary from 1 to 30 µm. Because fungal spores are smaller in diameter than pollens (approximately 1/1000), they can stay in the atmosphere for longer periods of time, and they can be carried farther distances by the wind (Kurup, 2000; Bush & Portnoy, 2001; D'Amato *et al*., 1997). All of the fungi that cause allergic diseases are saprophytc, and most of these fungi belong to the classes *Ascomycetes* and *Deuteromycetes* (Bush & Portnoy, 2001). Since fungal spores are small in diameter, they can reach te lower airways and cause both allergic rhinitis and allergic asthma (Chao *et al*., 2002; Wargocki *et al*., 2002).

The number and types of fungi in a region can vary depending on the geographic location, climate, and season. Moreover, fungal spores in the air can be affected by many factors, such as the wind, temperature, humidity, season, and rainfall. Although outdoor fungal spores are present in the atmosphere year-round, they increase in the atmosphere during certain periods like pollens (Kurup, 2000; Bush & Portnoy, 2001; D'Amato *et al*., 1997; Kilic, 2010).

Aerobiological studies enable us to ascertain the concentration of the fungal spores present in the atmosphere and give better understanding on inter-dependant relationship between their concentrations and meterological conditions. The aim of this study was to determine the relationships between the number and seasonal distribution of the fungal spores found in the paddy field atmosphere in Gondia district of Maharashtra, and the meteorological parameters around the paddy field.

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The inter-dependency between meteorological factors and the occurrence spores in the air during the 2-year period have been evaluated to understand the dynamics of these relationships to make aware the farmers to avoid such types of health risk during paddy growing season.

MATERIALS AND METHODS

Study area

The sites with full paddy plantation were selected for the study in Gondia district. The surveys and sampling have been carried over a plantation of rice species, *Oryza sativa* and variety, Jaishreeram.

Sampling

Collection of air fungal prop gules was undertaken with a volumetric air sampler (Hi-media air sampler) using Rose Bengal Agar strip. (i.e. impaction method) and Petri plate exposure (i.e. sedimentation method), using PDA and Czapekdoxe agar media. The study was carried out during two growing season June 2020 and June 2021. Sampling was performed once a week during both Kharif and Rabi seasons. The meteorological variables such as mean temperature and relative air humidity were measured at each sampling time and the data were confirmed with the daily forecasts issued by the local meteorological division and Meteorology website.

Classification and identification of Isolates

Petri dishes having fungal growth medium were incubated at 27°C for any contamination on growing medium. Incubated petri dishes without any contaminants were inoculated with the samples collected from the field. Inoculated petri dishes were incubated at 27°C for 7 days. The growing colonies were counted between 4 and 7, days of incubation. The data on number and concentration of fungi propagules detected in the air samples was recorded and calculated using the formula suggested by the Hi Media and expressed as unit of fungi concentration in air (cfu/m3). The identification of isolates genera was carried out by conventional methods.

Combination of two techniques viz. Hi-media air sampler (Impaction) and sedimentation method have been used in the present investigation to get a fairly complete picture of the air mycoflora of the rice field, In fact earlier worker emphasized the need of using two trapping methods during aerobiological surveys one for microscopic assessment of the total aerospora and other for the identification of predominant types in culture. (i.e. quantitative and qualitative analysis respectively).

Number and types of fungus spore in outdoor or field air seem to depend on direction of wind (Tilak, 1970). Hence combination of two techniques was used in the present investigation to get a fairly complete data of the air mycoflora of the rice field. Functional relationships between composition of airspora and meteorological factors were evaluated using Pearson correlation coefficient and two way ANOVA test.

RESULTS

The study suggested that at Dhakani site during Kharif season-I in 2020-2021 concentration of airborne

Table 1: Meteorological data recorded in the study area during 2020-21 and 2021-22. (A= Impaction, B = Sedimentation)

Month	Average Temperature (°C)		Average Relative Humidity (%)		Average Pressure mbar		Average rain fall (mm)		Average fungal Concentration with A (%)		Average fungal Concentration with B (%)	
	2020-21	2021-22	2020-21	2021-22	2020-21	2021-22	2020-21	2021-22	2020-21	2021-22	2020-21	2021-22
June	33	31	60	63	1000	1001	50.74	103.7	8.00	12.13	7.86	6.05
July	27	27	88	83	1001	1001	26.55	220.45	19.62	19.16	15.85	17.63
August	27	27	84	85	1002	1002	303.33	171.37	18.52	15.20	19.45	22.11
September	27	28	85	83	1004	1006	219.18	73.18	15.86	14.95	26.29	23.02
October	25	26	76	77	1009	1008	33.58	25.88	25.75	26.44	26.29	27.23
November	21	22	69	68	1014	1014	0.2	0.32	12.24	12.13	4.44	3.95
December	19	20	64	68	1015	1016	0.0	0.0	0.0	0.00	0.0	0.00
January	20	20	63	58	1014	1015	1.02	0.0	45.97	15.45	10.14	10.87
February	25	24	51	15	1009	1014	0.76	7.51	21.55	25.00	26.57	27.68
March	28	28	37	47	1005	1010	0.76	0.111	27.05	27.41	35.33	31.80
April	34	33	24	39	1003	1006	0.0	0.741	26.08	22.00	20.58	19.44
May	36	36	35	40	1001	1003	15.89	2.961	10.56	9.14	9.59	10.21

fungi the correlation between fungi and temperature was negative (r = -0364), when analysed using impaction method and whereas positive (r = 0.007) with sedimentation method. With relative humidity and air borne fungi the correlation was positive (r = 0.637 & r = 0.654 respectively) with both impaction and sedimentation method. Positive correlation of airborne fungi with average pressure was observed in same season (r = 0.13) for impaction method and negative correlation with sedimentation sample (r = -69). Correlation of average rainfall with total fungal concentration was r = 0.086, r = 0.487 for both impaction and sedimentation respectively (Table 1 & 2).

During Rabi season I (2020-2021), the Pearson correlation between temperature and average rainfall was negative obtained using both the methods (r = 0.805, r = - 0.076, & r = -0.662, r = -0.556 respectively). Whereas, correlation between relative humidity and average pressure was positive (r = 0.618; r = 0.835 respectively) among the samples obtained using impaction, while in with sedimentation the Pearson correlation was negative (r = -0.243, r = -0.110).

In the year 2021-22 the result of impaction method during Kharif Season- II, the value of pearson correlation between concentration of fungi and temperature was r = -0.364 and with relative humidity r = 0.634 with an average pressure (0.139) and average rainfall (r = 0.858). In case of Rabi season II correlation coefficient between temperature, relative humidity, average pressure, and rainfall was r = -0.805, r = 0.618 and 0.835 and -0.661 respectively (Tables 1&2).

Sample collected with sedimentation method during Kharif Season- II, had positive correlation with average temperature, relative humidity and average rainfall (r = 0.007, r = 0.654 and 0.488 respectively) and negative correlation with average pressure (r = -0.169). In case of Rabi season- II the Pearson correlation among Average temperature, Average relative humidity, Average pressure and Average rain was negative (r = -0.076, r = 0.2143, r = 0.110 and r = -0.550 respectively).

Predominant types of fungal spores

In the present study airborne fungal spore obtained majority of them are identified and shown in Fig.1(i-xii): i- *Aspergilli,* ii- *Alternaria,* iii- *Cladosporium,* iv- *Cercospora,* v- *Curvulari,* vi-*Fusarium,* vii- *Mucor,* viii - *Penicillium,* ix- *Rizopus,* x- *Tricoderma,* xi- *Torula,* xii- *Bipolaris and others such as Helminthisporium* and *Nigrospora etc.* The predominant fungal spores belonging to Dueteromycetes during Karif seasons I-II, with Pearson method, ratio ranged from 61.29-70.59%, comparatively low during Rabi season I-II 62.16-64.1%). Similarly with the Sedimentation methods 71.88-76.92 during Kharif and 71.43 during Rabi season I-II (Table 3). All the other spores classified under Ascomycetes (7.69-15.38), Oomycetes (0.0-16.71%), Zygomycetes (5.88-10.26 %), and the lowest in Basidiomycetes (0.0-7.69%) along all the seasons and at various climatic conditions (Table 3).

DISCUSSION

The aerobiological presence in paddy fields around Gondia a district of Maharashtra has been evaluated. The following relatively high incidences of pathological diseases, among its population, with airborne fungal spores being recognized as one of the significant offenders. During the present study, over 74 types of airborne fungal spore have been recorded. Fungal spores comprised a large portion of the aerospora of different fungal types as described (Mehta, 1952). Assam Aerospora over rice fields has been reported by number of workers. Singh (1989) reported that, most of the fungi affect rice cultivation and can spread through air. Chakrabarty *et al.* (2003) identified total of 26 fungal spores in the rice field in Kolkata.

In the present study it was observed that, when average temperature increased to 25-28^{o}C, the spore taxa and number of total spore counts reached their maximum. A Further gradual rise in temperature to 33-36^{o}C had a negative effect on the total spore taxa and spore counts.

Table 2: Pearson correlation between meteorological factors and airborne microorganism in the paddy field during 2020-2021 and 2021-22.

Season of Plantation	Average Temperature (^{0}C)		Average Relative Humidity (%)		Average Pressure		Average Rainfall in (MM)	
	2020-21	2021-22	2020-21	2021-22	2020-21	2021-22	2020-21	2021-22
Kharif-A	-0.36449496	0.212118912	0.636233594	0.394177734	0.138700454	-0.004710134	0.085760632	-0.020549593
Kharif-B	0.00748983	0.10741171	0.654248192	0.808364745	-0.169205456	-0.220924352	0.487884155	0.19283121
Rabi-A	-0.80508852	-0.331254867	0.617928165	-0.333639441	0.835378667	0.424147846	-0.661959434	0.105524807
Rabi- B	-0.07634373	-0.155547533	-0.243217626	-0.444748888	-0.11017885	0.280119135	-0.555885672	0.24040999

Table 3: Relative contribution of total fungal spore analysed using Impaction (A) Sedimentation (B) methods during 2020-21 and 2021-22.

Class of fungus	Kharif season (2020-21)		Rabi season (2020-21)		Kharif season (2021-22)		Rabi season (2021-22)	
	A (%)	B (%)	A (%)	B (%)	A (%)	B (%)	A (%)	B (%)
Deuateromyceates	70.59	76.92	64.1	71.43	61.29	71.88	62.16	71.43
Ascomyceates	11.76	7.69	15.38	10.71	25.8	9.38	13.51	10.71
Oomyceates	8.82	7.69	2.56	10.71	0.0	9.38	16.71	10.71
Zygomyceates	5.88	7.69	10.26	7.14	9.68	6.25	10.17	7.14
Basidiomyceates	2.94	0.0	7.69	0.0	3.29	3.13	3.57	0.0

The results of present study, on correlation of average maximum and minimum temperatures of total spore taxa as well as spore counts, it is clear that there is an optimum temperature range, which is favourable for fungal growth and spore production. The present findings are in accordance with the findings of Eversmeyer & Burleigh (1973). They suggested that sharp decrease in spore concentration were probably due to the reduced spore production when temperatures increases beyond an optimum suited for fungal growth.

The average of humidity exhibits an optimal range. Spore count get affected adversely when the average humidity rise beyond or falls below this optimal range, when average humidity was low or high, there was a decrease in the diversity of spore. In 2020-21 and 2021-22, the optimum humidity not showing relevance to the spore count. It is clear from the above result that, relative humidity is a single factor for the rise in the spore counts, as other weather parameters can also contribute to this phenomenon. Agarwal & Shivpuri (1974) have found, that relative humidity does not affect the spore concentrations independently in the atmosphere to a significant level.

Results of the present study show that the total monthly rainfall affected the spore taxa and total spore counts, increase in rainfall decreased the spore count. Low diversity and density of fungal spores in the atmosphere during rains have been reported by Mishra & Kamal (1971). Eversmeyer & Burleigh (1973) suggested that due to rain, most of the fungal spores washed away. There was marginal increase in the spore count just after the rains, in the month of September and October in kharif season during in both the years. This increase was mainly due to the presence of ascospore. Since the colored ascospores, non-septate and septate, were usually reach at their maximum diversity following the rain. However, according to Tilak and Srinivasulu (1970), the peak concentration of ascospores was September associated with heavy rain fall and high percentage of relative humidity. Levetin & Horowitz (1991) also stated that, ascospores are typically present in highest concentration during rains. The distribution and diversity of the fungal spores in the atmosphere vary, depending on the geographic location, climatic conditions, and season. The climate and other meteorological conditions have a significant influence on the amount and diversity of the fungal spores in the atmosphere.

The temperature was reported to have a positive effect on the release of fungal spores, thus increasing the concentration (Chakrabarti *et al.*, 2012). Akgul *et al.* (1916) reported the positive correlation between the temperature and the atmospheric spore levels of *Ascomycota*, *Basidiomycota*, *Epicoccum*, *Cladosporium*, *Alternaria*, and *Drechslera*. The changes in the fungal spores according to the meteorological parameters has positive correlation between the spore concentrations and the minimum and maximum temperatures and sunlight (Stepalska & Wolek, 1997).

Oliveira *et al.* (2005) demonstrated negative correlation between the fungal spore concentrations and the wind speed, but positive correlations between the fungal spore concentrations and the relative humidity and the temperature. Positive correlations between the atmospheric fungal spore concentrations and low relative humidity, a high wind speed, and a high air temperature, and negative correlations between the fungal spore concentrations and rainy weather and a high relative humidity have been reported (Calderon *et al*., 1995).

It was observed in the present findings that, when average pressure increased or decreased, affected the concentration of spore count in 2020-21, similar average pressure, was favourable for increase in fungal spore concentration during 2021-2022 during both Kharif and Rabi seasons (Table 3). Katial *et al.* (1997) performed series of analysis on the data of airborne fungal spore and correlated with meteorological factors. *Cladosporium* found to be positive with average daily temperature, relative humidity and negative with precipitation, The

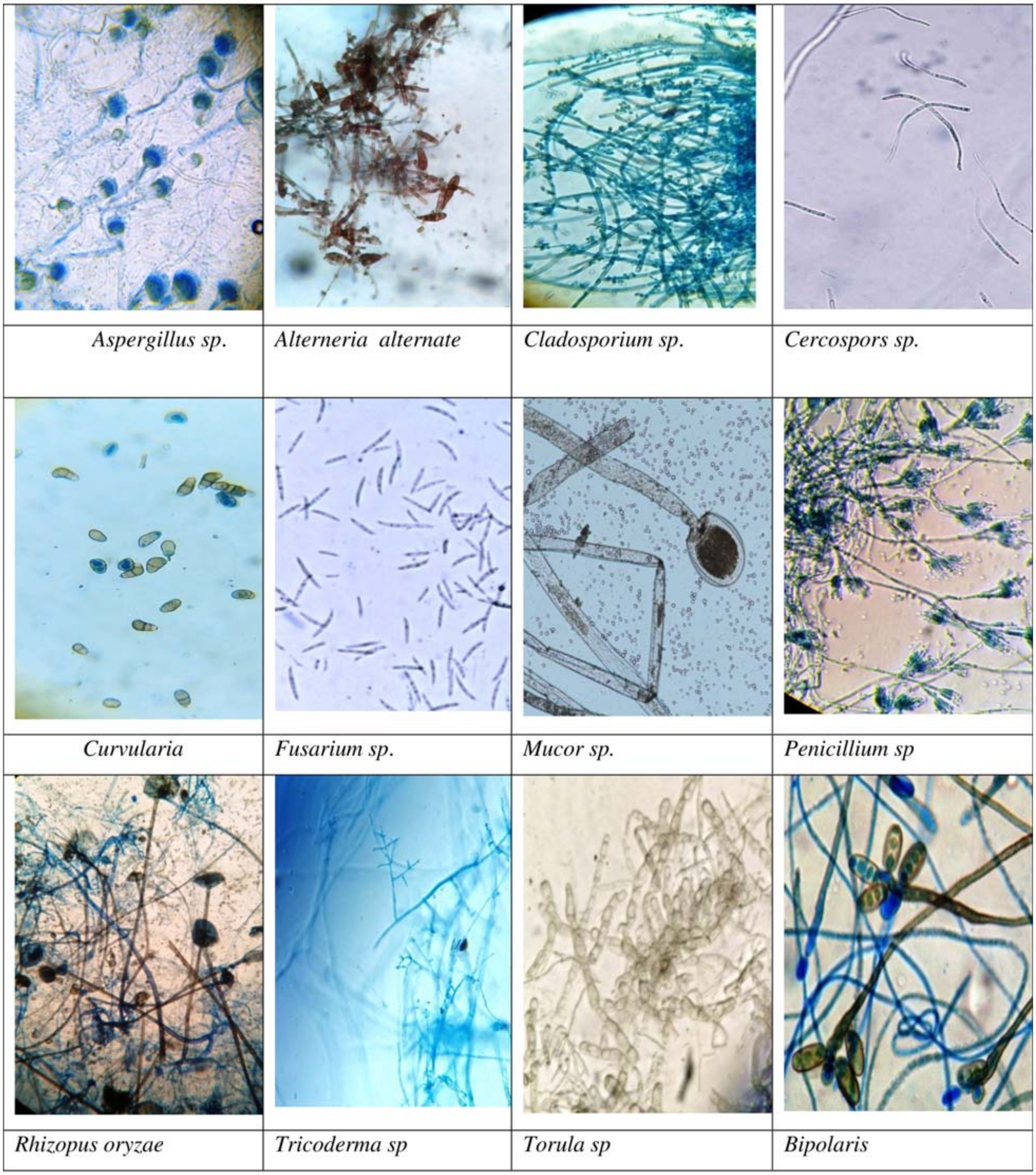

Fig. 1: Microscopic images of different fungus.

presesent results also showed positive correlation in C*ladosporium* and other species of fungi such as *Aspergilli, Penicillium*, *Rhizopus*, *Mucor*, *Alternaria*, *Fusarium* are with temperature and relative humidity.

Earlier studies have also indicated that, outdoor fungal spore concentration might have a positive correlation with temperature. Total fungal spore concentration in the air was not significantly influenced by relative humidity and rainfall. This may be due to fact that atmospheric parameters in the Indian sub-continent are highly fluctuating and inconsistent. Even during monsoon day to day rainfall differs. Our results have proved that prolonged rainfall influences the concentration of airborne spores resulting decrease in spore concentration during heavy rainfall.

CONCLUSION

Although the fungi and their spores are ubiquitous, the abundance of airborne spores shows distinct temporal patterns that persist across a mosaic of natural vegetation. Peak spore loads were found during the Kharif season, with lower spore abundance in the Rabi season. Across spatial and temporal dimensions, microclimate is a stronger indicator of airborne spore concentration than vegetation type. This study was the first aero fungal survey of the Gondia district, in Maharashtra, and new information has been introduced in the field of aerobiology.

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